Peptides

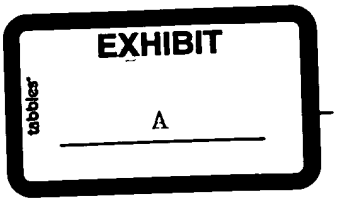
Chemistry and Biology

Proceedings of the Tenth American Peptide Symposium
May 23-28, 1987, St. Louis, Missouri, U.S.A.

Edited by

Garland R. Marshall
Department of Pharmacology
Washington University School of Medicine
St. Louis, Missouri 63110, U.S.A.

ESCOM ▪ Leiden ▪ 1988



A synthetic strategy for epitope mapping

H. Mario Geysen, Stuart J. Rodda and Tom J. Mason
Commonwealth Serum Laboratories, 45 Poplar Road, Parkville, Victoria 3052, Australia

Introduction

Synthetic peptides are attractive as probes for studying aspects of immunology at the molecular level, as possible diagnostic reagents, as characterized vaccine components and as hormone analogs. With the need for larger numbers of peptides for evaluation, fully automated synthesizers are widely used, and several methods have been described for the simultaneous synthesis of many peptides [1].

We describe a procedure allowing several thousand peptides to be concurrently synthesized at the rate of one residue coupled per day. With no practical restriction on the number of peptides which can be synthesized, a completely systematic approach to the location of epitopes (scan; Fig. 1) and their further resolution (replacement set; Fig. 2) becomes possible [2]. Good agreement has been shown between the results obtained from these rod-synthesized peptides and those from conventionally synthesized and purified peptides [3]. The stability of the rod-synthesized peptides on repeat testing allows their reuse for 30–60 tests.

This method has created the opportunity to address questions, which were formerly thought to be too difficult to answer, about the location of epitopes and their specificity for antibody.

Synthesis of Peptides

The method of peptide synthesis on rods has been described in detail elsewhere [2]. Briefly, specially-molded high-density polyethylene rods (diameter 4 mm) were suspended in deaerated 6% (v/v) acrylic acid in water containing 0.005 M $CuSO_4$. Gamma-irradiation at a dose of 0.8 Mrad was used to graft polymerize the monomer to the rods as polyacrylic acid. After a wash cycle, dried grafted rods were assembled into specially molded polyethylene holders designed to hold 96 rods in the format and spacing of a microtiter plate. Subsequent reactions at the tips of the rods were carried out in the wells of a specially molded polyethylene tray.

An amino group was introduced by reacting the polyacrylic acid on the rods for 2–3 days with mono-(Boc)-1,6-diaminohexane in DMF using DCC to achieve the condensation. Following a conventional Boc-deprotection cycle with TFA

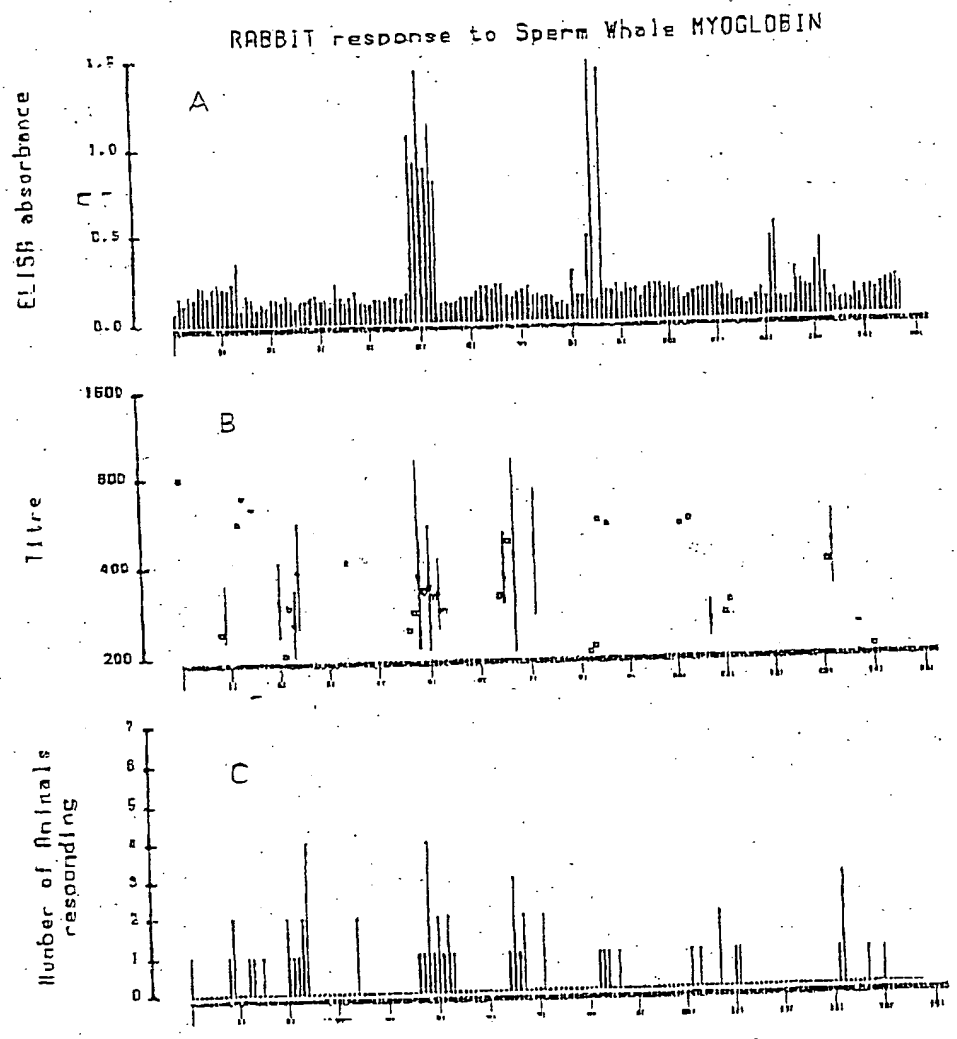


Fig. 1. Profiles of antigenic response (A–C) as a function of all possible sperm whale myoglobin (SWMb) hexapeptides. Each parameter is plotted at the sequence number corresponding to the first residue (amino-terminal) of the relevant hexapeptide. (A) Scan obtained from an individual rabbit anti-SWMb serum, used at a dilution of 1/400. The vertical axis shows the absorbance obtained at the end of the ELISA. (B) The individual titer (square) or, where more than one serum reacted, the geometric mean titer (circle) and the range of titers (vertical bar) of the antisera. Titers less than 200 (twice the slope of the test background) were ignored. (C) The frequency of the antigenic response given by the number of rabbit antisera that react with each hexapeptide.

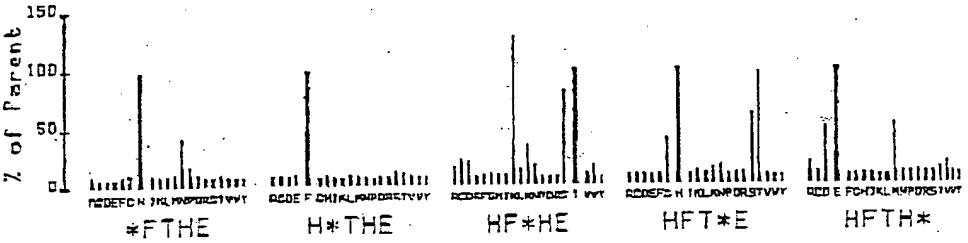


Fig. 2. Replacement set analysis, based on parent pentapeptide ^{54}HFTHE^{58} homologous with myohemerythrin. Each block of 20 ELISA values represents the results with peptides containing the single amino acid substitution identified by the single letter code beneath each bar. The position of the substitution is indicated by an asterisk in the sequence given under that block. The homologous amino acid is emphasized. ELISA values for the five copies of the parent sequence have been averaged and taken as 100% for the purposes of comparison with replacement analogs. Dilution of rabbit antiserum used was 1/1000.

Boc-β-alanine was coupled for a limited time to a target density of 50 ± 10 nmol per rod tip. Unreacted amino groups were acetylated by reaction with acetic anhydride in DMF/TEA. Successive DCC/HOBT-mediated coupling reactions were carried out overnight using side-chain protected amino acids as dictated by the sequence to be synthesized. A microcomputer program was used to calculate the requirements for the preparation of the activated amino acid solutions, and to direct the addition of the correct amino acid to each rod on each day. At the completion of the final coupling reaction, and after removal of the Boc group, the terminal amino group was acetylated. Side-chain deprotection was achieved by reaction with 50 mM boron tris(trifluoroacetate) in dry TFA [4].

Detection of Binding of Antibody by the Rod-Coupled Peptides

ELISA reactions were carried out with the appropriate serum and conjugate solutions in polystyrene microtiter trays [1]. Briefly, the tips of the rods, with the peptides still attached, were precoated using a 2% protein solution to block nonspecific adsorption of antibodies. Rods were incubated overnight in an appropriate dilution of an antiserum, washed 4 times, and incubated for 1 h in 'conjugate', comprising horseradish peroxidase-labeled goat anti-Ig, specific for the antibody species under test.

After washing to remove unbound conjugate, the level of bound conjugate was determined by the color developed by the reaction with a solution of the enzyme substrate, hydrogen peroxide in phosphate/citrate buffer, pH 4.0.

containing ABTS. Bound antibody was removed from the rods prior to retesting with another antiserum.

Rationale of Strategy

The procedure for the synthesis of peptides as described was conceived in order to provide the very large numbers of peptides required for systematic screening for sequences with biological activity. As such, the following factors were considered:

(1) By ELISA, the detection of binding of antibody only requires peptide to be present in the range of picomol [5]. This condition is satisfied by the level of peptide produced on the tips of the rods, which is typically 30–50 nmol. Furthermore, we have observed comparable test absorbances for peptide densities varying over two orders of magnitude, indicating that the test is only limited by the concentration of antibody.

(2) As in the case with the majority of serological tests, where a given antigen is determined in the presence of a large excess of extraneous protein, absolute purity of a peptide is also not a necessary requirement. The specificity of the antibody is relied upon to distinguish between the nominal sequence synthesized and the inevitable small amounts of deletion sequences, termination peptides, or other byproducts formed during the synthesis.

(3) Large numbers of 'negative control sequences' are a natural consequence of the systematic way in which peptide sets are structured. As is the usual case, peptide sets consist of closely related sequences differing by only one or two amino acids from each other. The observation of antibody binding to one peptide but failing to bind to a closely related peptide is taken as good evidence for the specificity of the test.

Stability on Repeat Testing

As a consequence of the synthesis strategy in which completed peptide remains covalently coupled to the plastic support, reuse of peptide through a number of tests only requires removal of reacting antibody between tests. When the absolute value of the absorbance is monitored over many successive tests with the same peptides, a gradual decrease is observed. Experience with many sets of peptides suggests that 30–60 useful tests can be expected before it becomes necessary to resynthesize a particular set of peptides. Peptides have been stored (dry at 4°C) for extended periods between tests, without detectable loss of activity.

Discussion

The possibility that synthetic peptides may find uses as vaccines, diagnostics

and other biologically relevant agents has focused interest on methods which provide for large numbers of peptides for evaluation. It is our experience that using the methods described, a small laboratory could readily prepare more than 1000 peptides per month. Furthermore, the format of these peptides makes them ideally suited for repeated evaluation, and utilizes the same instrumentation as is routinely used for immunological testing. Identification of a peptide(s) with the desired properties can then be followed by the preparation of larger and well-characterized qualities, using well-established solid phase synthesis procedures.

References

1. Houghten, R.A., Proc. Natl. Acad. Sci. U.S.A., 82 (1985) 5131.
2. Geysen, H.M., Rodda, S.J., Mason, T.J., Tribbick, G. and Schoofs, P.G., J. Immunol. Methods, in press.
3. Geysen, H.M., Tainer, J.A., Rodda, S.J., Mason, T.J., Alexander, H., Getzoff, E.D. and Lerner, R.A., Science, 235 (1987) 1184.
4. Pless, J. and Bauer, W., Angew. Chem., 85 (1973) 142.
5. Bittle, J.L., Houghten, R.A., Alexander, H., Shinnick, T.M., Sutcliffe, J.G., Lerner, R.A., Rowlands, D.J. and Brown, F., Nature, 298 (1982) 158.